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Mapping Quantitative Trait Loci for Resistance to Goss's Bacterial Wilt and Leaf Blight in North American Maize by Joint Linkage Analysis

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Abstract

Goss's wilt and leaf blight is a bacterial disease of maize (*Zea mays* L.) caused by the Gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis*. Goss's wilt has reemerged as an important disease in the western United States and is spreading to other areas. Although the reasons for this reemergence are not completely known, it is important to understand the genetic basis of resistance to Goss's wilt. The objective of this study was to map the quantitative trait loci (QTL) underlying resistance to Goss's wilt. To achieve this objective, joint linkage and linkage mapping in 3 of the 25 nested association mapping families were used. Three biparental linkage mapping families including 'B73' × 'Oh43', B73 × 'HP301', and B73 × 'P39' were evaluated for Goss's wilt in Nebraska. Eleven QTL were detected on chromosomes 1, 2, 3, 4, 5, and 10 through joint linkage mapping. The joint linkage model explained 45% of the phenotypic variation for Goss's wilt. Linkage mapping in each of the three families identified nine, six, and four QTL in the families B73 × Oh43, B73 × HP301, and B73 × P39, respectively. Joint linkage and linkage analysis were also conducted within each environment to detect any environment-specific QTL. However, most of the QTL were colocalized with QTL

detected in across environment joint linkage and linkage mapping. These results will help us to understand the genetic basis of resistance to Goss's wilt better and may facilitate maize breeding programs to incorporate resistance to Goss's wilt into the maize germplasm.

Abbreviations: NAM, nested association mapping; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; WMD, weighted mean disease

Clavibacter michiganensis subsp. *nebraskensis* is a Gram-positive bacterium causing Goss's wilt and leaf blight disease in maize (Vidaver and Mandel, 1974). Goss's wilt was first discovered in south central Nebraska in 1969 and quickly spread to different counties within Nebraska, as well as to Iowa, Kansas, Colorado, and South Dakota. In the disease evaluation trials, varying levels of resistance were found in maize inbred lines (Calub et al., 1974; Schuster et al., 1972). Partially resistant hybrids were developed and through the deployment of resistant hybrids over a period of 10 yr, the disease became sporadic, did not cause any severe damage, and occurred only in fields planted with susceptible maize hybrids (Jackson et al., 2007a; Vidaver et al., 1981). However, around 2006, Goss's wilt reemerged as an important disease in the North American Corn Belt (Jackson et al., 2007a). Since then, Goss's wilt has been observed in 60 counties in Nebraska (Jackson and Rees, 2010) and 80 counties in Iowa (Robertson, 2012). Goss's wilt has also been reported in Colorado, Illinois, Iowa, Kansas, Michigan, Minnesota, South Dakota, and Wisconsin (Centre for Agriculture and Biosciences International and European and Mediterranean Plant Protection Organization, 2000).

Goss's wilt infection can occur at any developmental stage, with the bacteria entering into the plant through leaves, stems, and roots (Schuster, 1975). There are two possible phases of Goss's wilt: (i) leaf blight and (ii) systemic wilt. Infection starts through wounds on the plant surface leading to water-soaked spots (or freckles), which progress to gray lesions and eventually lead to leaf blight. Less common systemic wilt occurs when the pathogen infects the vascular system and moves systemically through the xylem, leading to blockage of the vascular bundles. The systemic wilt phase is more common if infection occurs during early growth stages (Jackson et al., 2007a).

Loss in yield can be severe if Goss's wilt occurs on susceptible hybrids. Yield loss caused by Goss's wilt under artificial inoculation was estimated to be 44% in the susceptible maize hybrid 'A619' × 'A632' but the tolerant hybrid B73 × 'Mo17' yielded only 1% less than the control (Claflin et al., 1978). Similarly, yield loss was estimated to be 43.5% in a study including 42 related hybrids derived from the inbred lines A632 and A619. Yield reduction was found to be correlated with disease severity (Carson and Wicks, 1991). A significant reduction in yield was found in a susceptible sweet corn hybrid when inoculated at early as well as later growth stages, though yields of resistant hybrids were not affected. Disease incidence and severity depended on the level of resistance of the hybrid (Suparyono and Pataky, 1989a). Although recent yield losses caused by Goss's wilt are not well documented, it has been estimated that yield losses from Goss's wilt in Iowa, Illinois, Minnesota, and Nebraska totaled 0.878 Tg (Mueller and Wise, 2012).

Inheritance of resistance to Goss's wilt has been studied previously using classical techniques. Gardner and Schuster (1974) screened a panel of inbred lines and failed to find an

inbred line completely resistant to Goss's wilt. Evaluation of the F1, F2, and backcross generations involving these inbred lines led Gardner and Schuster (1974) to conclude that resistance to Goss's wilt is polygenic trait. In a subsequent study, Goss's wilt resistance again appeared to be a polygenic trait (Martin et al., 1975). Later studies using diallel mating designs and generation mean analysis indicated that additive variation accounted for most genotypic variation for Goss's wilt (Ngong-Nassah et al., 1992; Treat and Tracy, 1990).

Identification of strong associations between markers and resistance would be highly desirable, as phenotyping for Goss's wilt is laborious and prone to failure because of a variety of possible weather conditions, including hot and dry weather around inoculation. Moreover, a marker-QTL analysis would be the starting point for ultimately discovering the genes controlling resistance. To identify the chromosomal arms that possibly harbor the genes conferring resistance to Goss's wilt, Rocheford et al. (1989) screened 'M14' interchange stocks and found strong evidence of a genetic factor on chromosome arm 4S, but these authors were not able to rule out the presence of other resistance genes on other chromosome arms. Recently, in a genome-wide association study for different traits in historical Minnesota maize inbred lines, nine single nucleotide polymorphisms (SNPs) were found to be significantly associated with resistance to Goss's wilt that together explained 47% of the phenotypic variation (Schaefer and Bernardo, 2013).

Joint analysis of multiple QTL mapping populations with a common parent increases the power and precision of QTL detection and can better capture allelic variation in a comparatively diverse set of germplasm compared to single biparental mapping populations (Blanc et al., 2006). One such population is the nested association mapping (NAM) population of maize, in which 25 diverse founder lines were crossed with a common inbred line, B73 (Yu et al., 2008). Using the maize NAM population, numerous QTL with small additive effects have been identified for diseases of maize such as southern corn leaf blight and northern corn leaf blight (Kump et al., 2011; Negeri et al., 2011; Poland et al., 2011). These studies have greatly enhanced knowledge of the genetic architecture underlying these traits. Such studies are lacking for Goss's wilt, mainly because of this disease being sporadic in occurrence for many years. The recent reemergence of Goss's wilt, however, has increased interest in developing a better understanding of the genetic basis of resistance.

The objective of this study was to use joint linkage mapping to map the QTL underlying resistance to Goss's wilt in three diverse maize genetic backgrounds. The distribution of QTL effects among three types of maize was examined. The results from this study will provide knowledge on the genetic architecture underlying variation for resistance to Goss's wilt and will ultimately help to inform marker-based selection strategies and searches for resistance loci.

Material and Methods

Germplasm

Seed of F_5–derived recombinant inbred lines (RILs) from three families—B73 × Oh43, B73 × HP301, and B73 × P39—was obtained from the maize genetics stock center and increased through sib mating during the summers of 2011 and 2012 in Lincoln, Nebraska. These families of RILs were developed as a part of the NAM project (Yu et al., 2008). Each family

consisted of 200 RILs. The common parent, B73, is moderately resistant to Goss's wilt, but the other three parents are believed to be relatively susceptible. A second reason these parents were chosen is that the inbred lines Oh43, HP301, and P39 were derived from dent corn, popcorn, and sweetcorn backgrounds, respectively. The RILs have previously been genotyped with 1106 SNP markers (http://panzea.org, accessed 28 Apr. 2016).

Field Experiment

In 2012, 195 RILs from the B73 × Oh43 family were planted in a completely randomized design with one replication at O'Neill, Nebraska. The RILs were planted in single-row plots, 3.7 m long and 0.8 m apart. Inbred lines B73, Oh43, and A632 were planted as replicated checks to estimate the experimental error and make spatial adjustments as needed. Each check was replicated seven times.

In 2013, 172 RILs from the B73 × Oh43 family, 141 RILs from the B73 × HP301 family, and 125 RILs from the B73 × P39 family were planted at Mead, Nebraska. Most RILs were planted in one replication in a completely randomized design, except for 54 RILs from the B73 × Oh43 family, 53 RILs from the B73 × HP301 family, and 63 RILs from the B73 × P39 family. These RILs were replicated twice because extra field space and seeds were available. The inbred lines B73, Oh43, HP301, P39, and B14A were replicated six times each as checks throughout the experiment.

In 2014, 174 RILs from the B73 × Oh43 family, 143 RILs from the B73 × HP301 family, and 124 RILs from the B73 × P39 family were planted in a completely randomized design. As there was more interest in the dent types, the B73 × Oh43 family was replicated twice in 2014, but the other two families were planted in only one replication. The inbred line B14A (susceptible) was planted as a replicated check 23 times throughout the experiment to compare the success of inoculations and make spatial adjustments if necessary. The number of RILs across the years varied according to seed availability. In summary, the B73 × Oh43 family was planted and evaluated in 2012 (one replicate), 2013 (partially replicated), and 2014 (two replications). The B73 × P39 and B73 × HP301 families were evaluated in 2013 (partially replicated) and 2014 (one replicate).

Inoculation and Disease Rating

Clavibacter michiganensis subsp. *nebraskensis* isolates were maintained on nutrient broth agar media. The isolates were tested on the susceptible sweetcorn variety 'Golden Cross Bantam' in the greenhouse for pathogenicity before using them for inoculum preparation. Inoculum for field inoculations was prepared from five *Clavibacter michiganensis* subsp. *nebraskensis* isolates consisting of approximately 3 × 108 colony-forming units per mL. These isolates were collected as part of a multistate survey across the Midwest (Langemeier, 2012). Only isolates collected in Nebraska were used for field inoculations (225A, 225B, 225C, 10B, and 194C) according to Animal and Plant Health Inspection Service regulations. In 2012, inoculations for the B73 × Oh43 family at O'Neill, Nebraska, were performed by DuPont Pioneer using proprietary techniques. One disease rating was recorded at O'Neill 54 d after inoculation using a disease rating scale of 1–9 (Suparyono and Pataky, 1989b) on a whole-plot basis (Supplementary Fig. S1), where 1 represents a symptomless plot, 2 indicates disease spread within 5 cm of the point of inoculation, 3 indicates limited disease

spread over 5 cm from the point of inoculation, 4 indicates a large spread and lesions extending to the other end of the inoculated leaf, 5 indicates systemic infection with blight of uninoculated leaves; 6 indicates leaf blight and wilt, 7 and 8 indicate severe leaf blight and wilt, and 9 represents a completely dead plot. In 2013, inoculations were performed 49 d after planting when most of the plants were at the V6 stage of development. Plants were wounded using motorized weed whippers (curved shaft string trimmer, Model UT33600, Homelite Consumer Products Inc., Anderson, South Carolina). Inoculum was sprayed on the plants within 10 s after wounding using a backpack sprayer. Disease ratings were recorded 15 d and 30 d after inoculations using the rating scale described earlier. In 2014, inoculations were performed using the same method used in 2013, but the ratings were recorded 15, 30, and 45 d after inoculations.

Phenotypic Data Analysis

Weighted mean disease (WMD), which is equivalent to the standardized area under the disease progress curve (Balint-Kurti et al., 2010), was calculated for each environment separately. This was calculated as the average of two consecutive ratings multiplied by the number of days between the ratings. The values were then summed and finally divided by the total number of days of evaluation to obtain the WMD (Balint-Kurti et al., 2010). When only two ratings were taken, the mean of the two ratings is equal to WMD. Analysis of variance (ANOVA) was performed on the WMD values using PROC MIXED (SAS version 9.3, SAS Institute Inc., Cary, North Carolina). Assessment of the check performance in different areas of each field indicated that systematic spatial variation was not present and thus no spatial adjustments were applied. Least-squares means were calculated by fitting a model including environment and RIL as fixed effects. Pearson's correlation coefficients among environments were calculated using PROC CORR (SAS version 9.3). Heritability on a per-plot basis for Goss's wilt was estimated for each of the three families separately using PROC MIXED (SAS version 9.3) according to the method given by (Holland et al., 2003).

Linkage and Joint Linkage Mapping

Quantitative trait locus mapping was conducted using the WMD values for each plot and the SNP data on the three linkage mapping families. Joint stepwise regression, implemented in GLMSELECT (SAS version 9.3), was used to build a model of cofactors, where environment, family, and marker nested within family effects were fit as fixed effects (Buckler et al., 2009). The level of significance for effects to enter and remain in the model was set to p-values of 0.0001 and 0.0002, respectively. Cofactor selection was also conducted separately for each environment.

The entire genome was scanned using a window size of 20 cM with the cofactors identified in the model described above. One thousand permutations were used to determine the logarithm of odds threshold to maintain an experiment-wise error rate of 0.05 (Doerge and Churchill, 1996). The logarithm of odds threshold was determined to be 4.18. After identifying significant markers, significant allelic effects were tested for significance at $P < 0.05$ using a *t*-test comparing the alternative parent allele to the founder parent (B73) allele. To calculate the variation explained by each QTL, a general linear multiple regression model

was fitted with environment, family, and significant marker effects using PROC GLM (SAS version 9.3).

In addition, QTL mapping was performed on each linkage family separately across environments and within each environment. Instead of joint stepwise regression, as implemented earlier for all three families combined, a stepwise regression model was fitted with environment effects and marker effects only for each family using GLMSELECT (SAS version 9.3; Buckler et al., 2009). A one-dimensional scan of the genome was conducted in the same way as described above for the joint analysis. Linkage and joint linkage analyses were implemented using a SAS script previously available at the Buckler Lab website (www.maizegenetics.net, accessed August 2013).

Results and Discussion

Phenotypic Distribution

The phenotypic distribution of Goss's wilt was skewed toward resistance because of a lack of systemic disease development in most of the RILs (Fig. 1). This is likely to point toward the difficulty of establishing good artificial infection and the fact that the V6 (later) growth stage was targeted in the inoculation method used in this study. Infection at earlier growth stages through wounding of plants through hail and winds has been reported to cause severe Goss's wilt symptoms and yield losses (Jackson et al., 2007b). The inbred line B73 was found to be resistant compared to Oh43 and HP301. However, B73 and P39 did not differ significantly for Goss's wilt ratings. A genotype × environment interaction was found to be highly significant in the combined dataset ($P < 0.0001$) and for the B73 × Oh43 family ($P < 0.0001$) (Table 1). Variation caused by a genotype × environment interaction was not significant for the B73 × HP301 and B73 × P39 families. Earlier studies have reported significant genotype × environment interactions for Goss's wilt and have advised several years of testing when selecting genotypes for resistance to Goss's wilt (Carson and Wicks, 1991; Ngong-Nassah et al., 1992; Treat et al., 1990). Significant positive correlations were found among years for Goss's wilt WMD in all families. Correlations among the three environments for Goss's wilt ratings for B73 × Oh43 family ranged from $r = 0.63$ to 0.71 ($P < 0.0001$). Correlations for Goss's wilt ratings between the two environments were also significant for the B73 × HP301 family ($r = 0.60$, $P < 0.0001$) and the B73 × P39 family ($r = 0.61$, $P < 0.0001$). High positive correlations indicated consistency in disease development across environments despite the skewed distribution of phenotypes and hence the data were combined across environments for the QTL analysis as well as being analyzed separately. Carson and Wicks (1991) also observed high correlations between Goss's wilt ratings recorded in different years despite the presence of a hybrid × environment interaction. Heritability estimates on an individual plot basis for Goss's wilt were high and very similar across the three families, ranging only from 0.60 to 0.62. In a single-year trial of F_2 populations, broad-sense heritability estimates for Goss's wilt were also high (0.63–0.80) in resistant × susceptible crosses. Heritabilities have been reported to be lower in intermediate × susceptible crosses (0.21–0.33) (Ngong-Nassah et al., 1992).

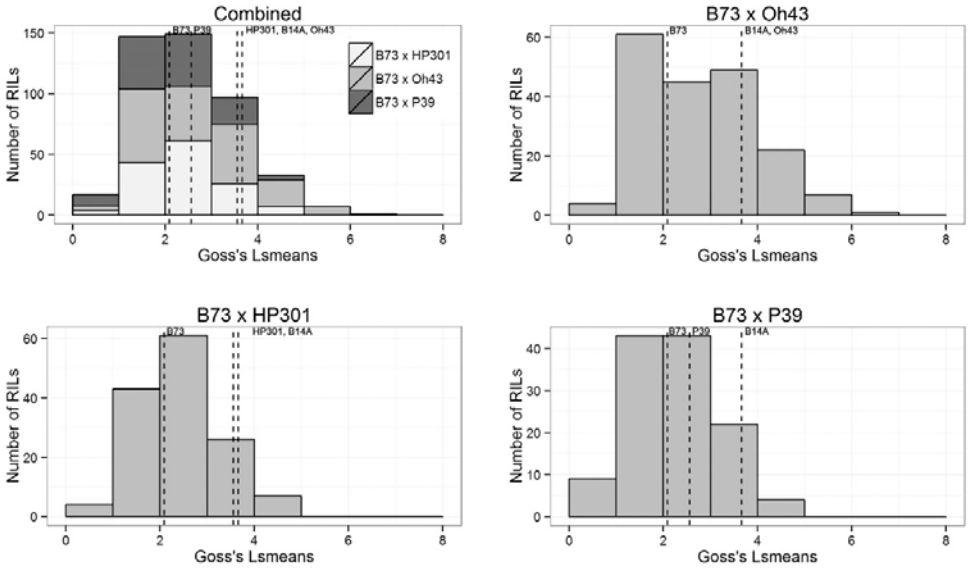

Figure 1. Histograms showing distributions of the least-squares means of Goss's wilt recombinant inbred lines of maize. Both combined and individual family distributions are displayed.

Table 1. ANOVA of Goss's wilt infection data of all three families of maize (B73 × Oh43, B73 × HP301, and B73 × P39) combined, as well as independently

Source of variation	df	Mean square	*F*-value	*P*-value
Combined dataset				
Environment	2	45.07	139.29	< 0.0001
Family	2	4.77	14.75	< 0.0001
RIL (family)	448	2.95	9.12	< 0.0001
RIL (family) × Environment	530	0.70	2.15	< 0.0001
Residual	279	0.32	—	—
B73 × Oh43				
Environment	2	27.96	87.31	< 0.0001
RIL	188	5.00	15.63	< 0.0001
RIL × environment	325	0.88	2.75	< 0.0001
Residual	185	0.32	—	—
B73 × HP301				
Environment	1	35.51	143.75	< 0.0001
RIL	140	1.33	5.40	< 0.0001
RIL × environment	113	0.36	1.46	0.0802
Residual	42	0.25	—	—
B73 × P39				
Environment	1	4.35	10.96	0.0017
RIL	120	1.61	4.06	< 0.0001
RIL × environment	90	0.38	0.96	0.5682
Residual	52	0.40	—	—

Linkage and Joint Linkage Mapping

This study is the first to report QTL for Goss's wilt resistance using linkage mapping techniques. Linkage and joint linkage mapping across environments detected 11 QTL controlling resistance to Goss's wilt (Fig. 2; Table 2). The allelic effect estimates were small, especially in joint linkage mapping, where no allelic effect was greater than 0.5 on a rating scale of 1 to 9 (Fig. 3). Previous studies using diallel, generation means analysis and chromosomal interchange stocks indicated that the inheritance of resistance to Goss's wilt is polygenic (Ngong-Nassah et al., 1992; Rocheford et al., 1989; Treat and Tracy, 1990). The results from this study, as well as those reported by Schaefer and Bernardo (2013), are in accordance with this hypothesis, as each QTL identified explained only a small amount of phenotypic variation, ranging from 1 to 6% (Table 2).

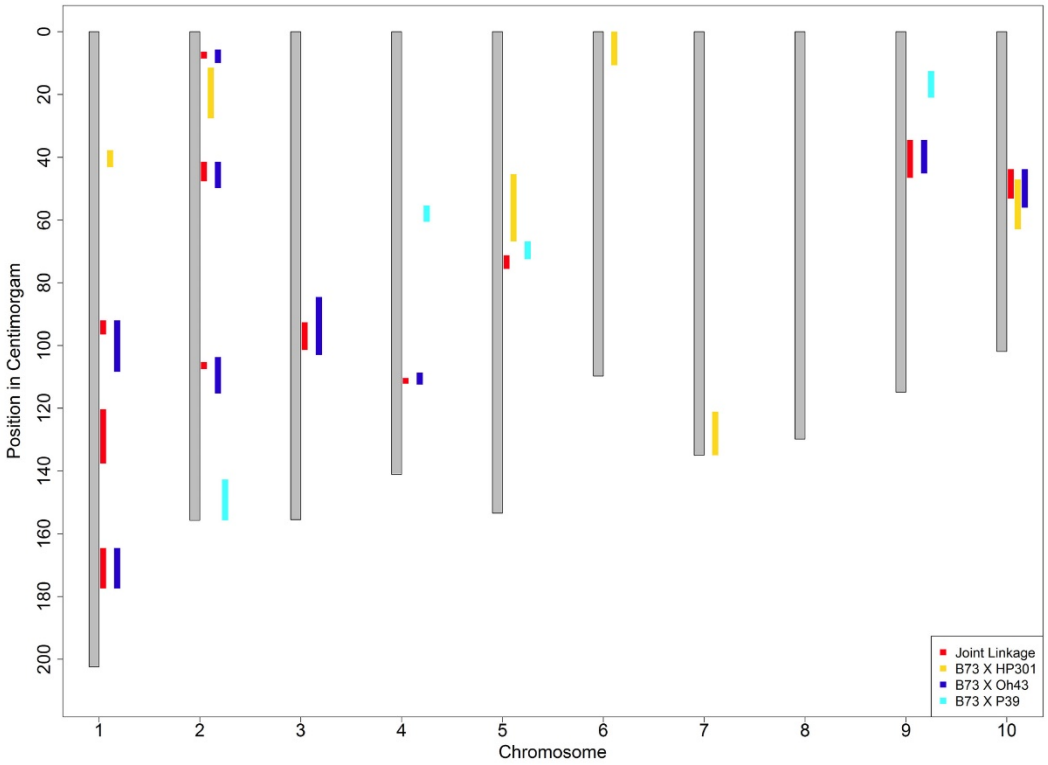

Figure 2. Comparison of quantitative trait locus (QTL) positions from joint linkage mapping and linkage mapping in each family. Ten maize chromosomes are shown as vertical gray bars. Segments of different colors indicate mapped QTLs at that position either identified using joint linkage or linkage mapping. The lengths of the segments show the range of the two logarithm of odds (2-LOD) support interval of the QTLs.

Table 2. Significant genetic markers from joint linkage mapping and linkage mapping in each family of maize conducted across environments

						Additive effect‡‡		
Marker†	Chr‡	Pos§ (cM)	LOD¶	2-LOD# (cM)	R^2††	Oh43	HP301	P39
Joint linkage mapping								
an1.5	1	94.9	29.5	92.0–96.5	0.06	–0.433	–0.060	–0.238
PHM4942.12	1	134	5.3	120.3–137.6	0.01	–0.166	0.157	0.030
PZA02957.5	1	176.9	11.4	164.6–177.5	0.02	0.222	0.160	0.216
PZA00902.1	2	6.8	16.6	6.4–8.5	0.03	–0.328	–0.083	–0.008
PZA03559.1	2	41.8	18.7	41.5–47.7	0.04	0.368	–0.094	–0.018
PZA02017.1	2	106.2	14.1	105.3–107.5	0.03	0.273	–0.088	0.206
PZA00494.2	3	97.8	13.0	92.6–101.4	0.03	–0.384	0.108	–0.018
PZA02479.1	4	111.3	20.0	110.4–112.2	0.05	0.306	0.302	0.101
PZB01017.1	5	74.5	15.6	71.3–75.6	0.03	–0.144	–0.349	–0.295
PZB00547.3	9	40	7.8	34.5–46.6	0.02	0.201	0.050	0.125
PZA03196.1	10	48.8	18.3	43.8–53.2	0.04	0.257	0.330	0.099
Linkage mapping (B73 × Oh43)								
an1.5	1	94.9	30.8	92.0–108.4	0.10	–0.502		
PZA02204.1	1	171.4	7.6	164.6–177.5	0.03	0.254		
PZA00902.1	2	6.8	14.0	5.7–10.0	0.05	–0.330		
PZA03559.1	2	41.8	18.7	41.5–49.8	0.06	0.412		
PHM3668.12	2	106.1	9.8	103.7–115.3	0.04	0.279		
PHM824.17	3	100.5	10.4	84.6–103.0	0.04	–0.462		
PZA02479.1	4	111.3	14.2	108.7–112.5	0.06	0.279		
PZB00547.3	9	40	6.2	34.5–45.2	0.02	0.345		
PZA00647.9	10	52.2	6.1	43.8–56.1	0.02	0.209		
Linkage mapping (B73 × HP301)								
PHM4531.46	1	39.7	7.9	37.8–43.2	0.07	0.268		
PZA03747.1	2	22.6	5.4	11.5–27.6	0.05	–0.226		
PZA00934.2	5	56.1	5.4	45.4–66.8	0.05	–0.237		
PHM15961.13	6	0	4.0	0.0–10.7	0.03	0.189		
PZA02274.1	7	135	5.7	121.1–135.0	0.05	0.226		
PZA01005.1	10	49.2	6.1	47.1–63.0	0.05	0.247		
Linkage mapping (B73 × P39)								
PZA03577.1	2	154.9	5.1	142.7–155.7	0.06	0.263		
PZA03203.2	4	57.4	5.0	55.4–60.6	0.06	–0.289		
PZA01779.1	5	68.1	14.3	66.8–72.5	0.19	–0.494		
PZA00466.1	9	20.7	4.8	12.6–21.0	0.06	0.249		

† Marker name as listed on nested association mapping map in cM
‡ Chromosome
§ Map position of each marker on the chromosome
¶ Logarithm of odds score
# Two-logarithm of odds (2-LOD) support interval in cM
†† Variation explained by each marker
‡‡ Additive effect estimates of the alleles from each parent

Single-family linkage mapping in the B73 × Oh43 family identified nine QTL on chromosomes 1, 2, 3, 4, 9, and 10. In the B73 × HP301 family, six QTL were detected on chromosomes 1, 2, 5, 6, 7, and 10. In the B73 × P39 family, four QTL were detected on chromosomes 2, 4, 5, and 9 (Fig. 2). As a comparison, all QTL detected via linkage mapping in the B73 × Oh43 family were also detected with joint linkage mapping. However, linkage mapping in the B73 × HP301 family detected QTL on chromosomes 6 and 7 that were not found using joint linkage mapping. Similarly, four QTL on chromosomes 1, 2, 4, and 9 were detected using single-family linkage mapping but were not detected using joint linkage mapping (Fig. 2). The statistical significance of these QTL just exceeded the thresholds in the single-family analysis, though their statistical significance in the joint linkage analysis was just below the threshold. This can probably be attributed to the lack of an effect at these positions within the B73 × Oh43 family, which was the largest family and was evaluated in the most environments and thus contributed the most data. The absence of an effect could have diluted the effect within the smaller families, resulting in a lack of significance in these few borderline cases. The unbalanced nature of the data in this study makes it difficult to find an exact explanation.

All but two of the QTL were detected using the across-environment analysis, which was expected on the basis of the high correlations between environments for Goss's wilt ratings. Two additional QTL were detected when the analyses were performed on single environment, but these QTL were small (R^2 = 0.04 and 0.07) and were only detected using single-family analyses (Supplementary Table S1). This result suggests these QTL are stable across environments and that perhaps QTL for Goss's wilt resistance detected in general show little interaction with the environment.

The motivation for including sweetcorn and popcorn parents was to find alleles that make sweet corn and popcorn susceptible to Goss's wilt compared to B73 but we found that all parents contributed alleles conferring both resistance and susceptibility (Fig. 3, Table 2, Supplementary Table S1). Allelic effect estimates of the QTL were positive at some loci but negative at others, indicating that B73, although relatively resistant, carries alleles for susceptibility to Goss's wilt (Fig. 3, Table 2). For example, the allelic effect of B73 is negative (susceptible) on chromosome 1 at 94.9 cM and positive (tolerant) on chromosome 4 at 111.3 cM (Fig. 3). This was expected, as transgressive segregation was observed in each family. On chromosome 1 at 134 cM, the B73 allele had a positive effect in the B73 × Oh43 family but a negative effect in the B73 × HP301 family (Fig. 3). This indicates the presence of an allelic series and possibly a "common gene-rare variant" situation as observed for other traits in the maize NAM (Wallace et al., 2014).

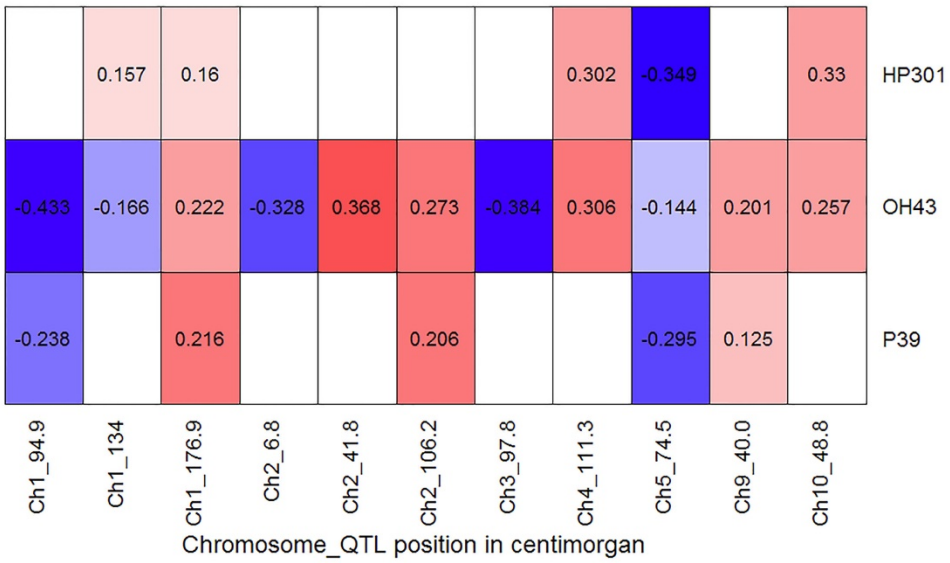

Figure 3. Heat map of allelic effect estimates for the B73 allele and founder maize parents from joint linkage mapping across environments. Positive (red) effects indicate that the B73 allele confers increased resistance and negative (blue) effects indicate that the B73 allele contributes to susceptibility. Only allelic effects that are significantly different from zero at the 5% significance threshold level are colored.

In conclusion, we report several QTL associated with resistance to Goss's wilt and their allelic effects across three distinct genetic backgrounds. Both joint linkage and linkage mapping helped in identification of the QTL. The QTL may be useful to maize breeders attempting to introgress resistance to Goss's wilt into elite lines used in dent corn, popcorn, and sweet corn breeding.

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References

Balint-Kurti, P.J., J. Yang, G. Van Esbroeck, J. Jung, and M.E. Smith. 2010. Use of a maize advanced intercross line for mapping of QTL for northern leaf blight resistance and multiple disease resistance. Crop Sci. 50:458–466. doi:10.2135/cropsci2009.02.0066

Blanc, G., A. Charcosset, B. Mangin, A. Gallais, and L. Moreau. 2006. Connected populations for detecting quantitative trait loci and testing for epistasis: An application in maize. Theor. Appl. Genet. 113:206–224. doi:10.1007/s00122-006-0287-1

Buckler, E.S., J.B. Holland, P.J. Bradbury, C.B. Acharya, P.J. Brown, C. Browne, et al. 2009. The genetic architecture of maize flowering time. Science 325:714–718. doi:10.1126/science.1174276

Centre for Agriculture and Bioscience International and European and Mediterranean Plant Protection Organization. 2000. *Clavibacter michiganensis* subsp. *nebraskensis* [distribution map]. Map No. 549. CAB International, Wallingford, UK.

Calub, A., W.A. Compton, C.O. Gardner, and M.L. Schuster. 1974. Reaction of 113 corn inbreds to leaf freckles and wilt of corn. Plant Dis. Rep. 58:956–960.

Carson, M.L., and Z.W. Wicks. 1991. Relationship between leaf freckles and wilt severity and yield losses in closely related maize hybrids. Phytopathology 81:95–98. doi:10.1094/Phyto-81-95

Claflin, L.E., D.L. Bockelman, E.A. Shahin, and T.L. Walter. 1978. The effect of *Corynebacterium nebraskense* on corn yields. Phytopathology News 12:86.

Doerge, R.W., and G.A. Churchill. 1996. Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294.

Gardner, C.O., and M.L. Schuster. 1974. Genetic studies of susceptibility to bacterial leaf freckles and wilt, *Corynebacterium nebraskense*. Maize Genet. Coop. News Lett. 47:155–157.

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breed. Rev. 22:9–112.

Jackson, T.A., R.M. Harveson, and A.K. Vidaver. 2007a. Goss's bacterial wilt and leaf blight of corn. NebGuide. G1675. Univ. of Nebraska–Lincoln Extension, Inst. of Agriculture and Natural Resources, Lincoln, NE.

Jackson, T.A., R.M. Harveson, and A.K. Vidaver. 2007b. Reemergence of Goss's wilt and blight of corn to the central high plains. Plant Health Prog. doi:10.1094/PHP-2007-0919-01-BR

Jackson, T.A., and J. Rees. 2010. Corn diseases update: Southern rust confirmed and Goss's wilt continues. CropWatch Newsletter August: 6–13.

Kump, K.L., P.J. Bradbury, R.J. Wisser, E.S. Buckler, A.R. Belcher, M.A. Oropeza-Rosas, et al. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat. Genet. 43:163–168. doi:10.1038/ng.747

Langemeier, C.B. 2012. Improved understanding of factors influencing the re-emergence of Goss's bacterial wilt and blight of corn. MS thesis, Univ. of Nebraska–Lincoln, Lincoln, NE.

Martin, P.R., C.O. Gardner, A.G. Calub, and M.L. Schuster. 1975. Inheritance of susceptibility and tolerance to leaf freckles and wilt (*Corynebacterium nebraskense*) of corn. Maize Genet. Coop. News Lett. 49:137–138.

Mueller, D., and K. Wise. 2012. Corn disease loss estimates from the United States and Ontario, Canada. Purdue Extension Publication BP-96-12-W. Purdue Univ., West Lafayette, IN.

Negeri, A.T., N.D. Coles, J.B. Holland, and P.J. Balint-Kurti. 2011. Mapping QTL controlling southern leaf blight resistance by joint analysis of three related recombinant inbred line populations. Crop Sci. 51:1571–1579. doi:10.2135/cropsci2010.12.0672

Ngong-Nassah, E.N., M.L. Carson, and Z.W. Wicks. 1992. Inheritance of resistance to leaf freckles and wilt caused by *Clavibacter michiganense* subsp. *nebraskense* in early maturing maize inbred lines. Phytopathology 82:142–146. doi:10.1094/Phyto-82-142

Poland, J.A., P.J. Bradbury, E.S. Buckler, and R.J. Nelson. 2011. Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc. Natl. Acad. Sci. USA 108:6893–6898. doi:10.1073/pnas.1010894108

Robertson, A.E. 2012. Goss's wilt: A 2012 recap and looking ahead to 2013. Proceedings of the 2013 Wisconsin Crop Management Conference 52, Madison, WI. 15–17 Jan. 2013. Soil Science Extension, Univ. Wisconsin-Madison, Madison WI. p. 172–174.

Rocheford, T.R., C.O. Gardner, and A.K. Vidaver. 1989. Genetic studies of resistance in maize (*Zea mays* L.) to Goss's bacterial wilt and blight (*Clavibacter michiganensis* subsp. *nebraskensis*). J. Hered. 80:351–356.

Schaefer, C.M., and R. Bernardo. 2013. Genome-wide association mapping of flowering time, kernel composition, and disease resistance in historical Minnesota maize inbreds. Crop Sci. 53:2518–2529. doi:10.2135/cropsci2013.02.0121

Schuster, M.L. 1975. Leaf freckles and wilt of corn incited by *Corynebacterium nebraskense* Schuster, Hoff, Mandel, Lazar 1972. Univ. Nebraska–Lincoln Research Bulletin 270. Inst. of Agriculture and Natural Resources. Univ. of Nebraska–Lincoln, Lincoln, NE.

Schuster, M.L., W.A. Compton, and B. Hoff. 1972. Reaction of corn inbred lines to the Nebraska leaf freckles and wilt bacterium. Plant Dis. Rep. 56:863–865.

Suparyono, and J.K. Pataky. 1989a. Relationships between incidence and severity of Stewart's and Goss's bacterial wilts and yield of sweet corn hybrids. Crop Prot. 8:363–368.

Suparyono, and J.K. Pataky. 1989b. Influence of host-resistance and growth stage at the time of inoculation on Stewart wilt and Goss wilt development and sweet corn hybrid yield. Plant Dis. 73:339–345.

Treat, C.L., and W.F. Tracy. 1990. Inheritance of resistance to Goss's wilt in sweet corn. J. Am. Soc. Hortic. Sci. 114:672–674.

Treat, C.L., W.F. Tracy, P.N. Drolsom, and J.G. Coors. 1990. Inheritance of resistance to Goss's wilt in maize. Crop Sci. 30:893–896. doi:10.2135/cropsci1990.0011183X003000040027x

Vidaver, A.K., and M. Mandel. 1974. *Corynebacterium nebraskense*, a new, orange-pigmented phytopathogenic species. Int. J. Syst. Bacteriol. 24:482–485. doi:10.1099/00207713-24-4-482

Vidaver, A.K., D.C. Gross, D.S. Wysong, and B.L. Doupnik. 1981. Diversity of *Corynebacterium nebraskense* strains causing Goss's bacterial wilt and blight of corn. Plant Dis. 65:480–483. doi:10.1094/PD-65-480

Wallace, J.G., S.J. Larsson, and E.S. Buckler. 2014. Entering the second century of maize quantitative genetics. Heredity 112:30–38. doi:10.1038/hdy.2013.6

Yu, J., J.B. Holland, M.D. McMullen, and E.S. Buckler. 2008. Genetic design and statistical power of nested association mapping in maize. Genetics 178:539–551. doi:10.1534/genetics.107.074245

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Supplementary Table S1. Significant genetic markers from joint linkage mapping and linkage mapping in each family conducted for each environment/year separately. The columns from left to right display marker name, chromosome, map position of each maker on the chromosomes in centimorgan (cM), logarithm of odds (LOD), and 2-LOD support interval in cM, variation explained by each term (R^2), and additive effect estimate of alleles.

[a]Marker	[b]Chr	[c]Pos (cM)	[d]LOD	[e]2-LOD (cM)	[f]R^2	[g]Additive effect		
						Oh43	HP301	P39
PZA02393.2	1	33.1	7.1	31.7–37.8	0.03	–0.192	0.244	0.041
PZA02823.1	1	133.9	7.4	129.8–134.8	0.03	–0.251	0.095	0.094
PZD00022.5	2	155.7	7.6	152.5–155.7	0.04	–0.048	–0.160	0.316
PZA03647.1	3	96.9	15.9	92.6–103.2	0.08	–0.453	0.080	–0.157
PZA01926.1	4	69.8	7.3	61.8–76.2	0.03	0.200	–0.125	–0.428
PZA00155.1	4	111.5	8.3	102.9–112.2	0.04	0.213	0.306	0.109
PZA00067.10	5	72.5	21.9	68.7–74.5	0.11	–0.159	–0.353	–0.596
PZA00758.1	8	49.9	5.8	42.0–52.4	0.03	0.258	–0.018	0.232
PZA00466.1	9	20.7	6.6	18.7–28.5	0.03	0.314	0.079	0.130
PZA02398.2	10	43.4	6.8	40.6–53.2	0.03	0.257	0.233	0.023
Joint linkage mapping 2014								
an1.5	1	94.9	17.9	92.0–108.4	0.12	–0.445	–0.078	–0.255
PZA00894.7	1	180.9	6.3	169.2–188.2	0.04	0.236	0.157	0.200
PZA03559.1	2	41.8	5.4	38.6–62.2	0.03	0.271	–0.108	0.058
PZB00772.7	2	117.5	4.2	109.9–125.9	0.03	0.186	–0.162	0.177
PZB01017.1	5	74.5	7.0	72.5–75.6	0.04	–0.233	–0.228	–0.223
PZA03196.1	10	48.8	7.7	44.8–53.2	0.05	0.168	0.387	0.152
Linkage mapping B73 x Oh43 2012								
PZA00455.14	1	96.5	9.6	89.6–108.4	0.15	–0.716		
PZA00497.4	2	49.8	5.1	41.5–64.2	0.07	0.513		
PHM3637.14	4	92.7	6.2	81.9–102.6	0.09	0.560		
PZA02128.3	10	44.8	7.4	42.9–53.2	0.11	0.642		
Linkage mapping B73 x Oh43 2013								
PZA03228.4	2	50.8	4.1	41.5–58.8	0.06	0.290		
PHM824.17	3	100.5	9.5	96.7–103.2	0.15	–0.456		
PZA03275.4/1	4	85.2	5.4	81.7–89.1	0.08	0.333		
PZA00416.7	8	20.7	4.7	10.5–32.1	0.07	0.309		
Linkage mapping B73 x Oh43 2014								
an1.5	1	94.9	16.7	92.0–98.4	0.18	–0.478		
PZA00978.1	1	177.5	3.6	164.6–191.5	0.03	0.210		
PZA01211.1	2	10.0	4.2	0–22.6	0.04	–0.232		
PZA03559.1	2	41.8	8.0	41.5–49.8	0.08	0.352		
PZA00494.2	3	97.8	5.9	92.6–101.4	0.06	–0.273		
PZA03645.1	7	49.4	3.4	47.8–63.7	0.03	0.208		
Linkage mapping B73 x HP301 2013								
PHM4531.46	1	39.7	6.5	29.9–43.2	0.10	0.311		
PZA01935.10	2	19.5	3.5	7.1–27.6	0.05	–0.232		
PZA00155.1	4	111.5	7.1	110.4–112.2	0.11	0.341		
PZA01796.1	5	75.6	7.9	71.3–78.4	0.13	–0.359		
PZA00048.1	10	42.9	6.8	38.6–46.7	0.11	0.322		
Linkage mapping B73 x HP301 2014								
PZA03274.4	5	50.8	3.6	45.4–66.8	0.10	–0.30808		

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PZA03713.1	10	48.0	5.4	44.8–58.4	0.16	0.370403
Linkage mapping B73 x P39 2013						
PZA01735.1	2	91.5	4.2	85.5–105.3	0.08	0.310686
PZA03203.2	4	57.4	5.1	55.4–75.3	0.09	–0.35472
PZA01779.1	5	68.1	9.3	66.8–74.5	0.19	–0.47276

a Marker name as listed on NAM map in centimorgam

b Chromosome

c Map position of each marker on the chromosome

d Logarithm of odds score

e 2-LOD support interval in centimorgan

f Variation explained by each marker

g Additive effect estimates of alleles from each parent


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